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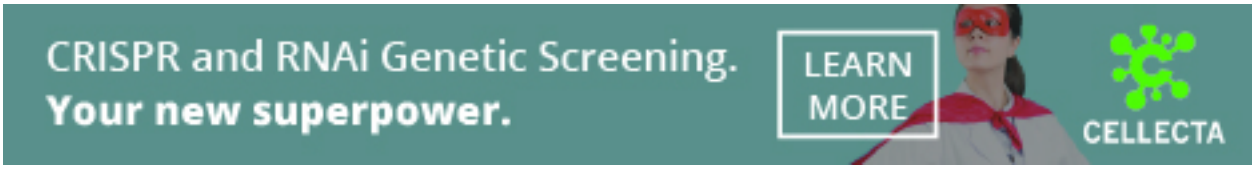

RESEARCH

Mapping of the OB Receptor to 1p in a Region of Nonconserved Gene Order from Mouse and Rat to Human

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As part of an effort to identify informative molecular markers for genetic analysis of human pedigrees segregating for obesity, we have developed a genetic map of human 1p in the region of the OB receptor (OBR), the gene that is defective in murine *diabetes* (Obr^{db}) and rat Zucker *fatty* (Obr^{fa}) mutations located on mid-chromosome 4 and chromosome 5, respectively. OBR was mapped 0.9 cR centromeric to WI-9515 and 2.2 cR telomeric of WI-7249 by radiation hybrid (RH) mapping. Ten yeast artificial chromosomes (YACs) containing OBR were identified, confirming the location of OBR centromeric to WI-9515 and telomeric to WI-7249. Additionally, five PI artificial chromosomes (PACs) were identified that comprised a contiguous series of overlapping clones spanning the length of OBR. WI-5182 was contained within the two PACs that are 3′ of OBR. Using a panel of 68 individuals from a single three-generation family and an additional nuclear family, we have mapped 18 polymorphic markers including phosphoglucomutase 1 (PGM1), which is centromeric to Obr^{db}/Obr^{fa}, and DIS85, which is telomeric to Obr^{db}/Obr^{fa} in the mouse and rat. The following composite map integrates these radiation hybrid, genetic, and physical maps: Centromere-//WI-7249–[OBR;WI-5182]–DIS198–[WI-9515;WI-6550;DIS2866]–DIS2825–[WI-3077; DIS2886]–[DIS515;DIS613;PGM1]–[DIS312;DIS473;DIS230;DIS246;DIS203]–DIS1643–[DIS1669;DIS1596;]UNC]–DIS476–DIS85–DIS220–C8B–GTAT1A7. Unresolvable markers are within brackets. A comparison of gene order on mouse chromosome 4, rat chromosome 5, and human 1p indicates that between rodents and humans, there has been a rearrangement of the gene order in the region surrounding OBR.

Obesity is a highly prevalent (Martin et al. 1995) and very costly (Colditz 1992) problem in industrialized societies. The phenotype involves predisposing genes, environmental, and developmental factors (Bouchard 1991).

Heritability for measures of obesity-related phenotypes including body mass index (BMI), amount of subcutaneous fat, percentage body fat, regional fat distribution, and weight gain in response to overfeeding has been estimated to be between 0.25 and 0.80 (Bouchard 1988a,b; Bouchard and Perusse 1993a,b). Similarly, the concordance of BMI and body fat content is nearly twice as high in monozygous compared to dizygous twins, and is correlated more highly among biological than adopted siblings (Stunkard et al. 1986; Sorensen et al. 1989, 1990; Price and Gottesman 1991; Sorensen 1995). The specific genes underlying these effects on body fat composition in humans have yet to be determined.

Within the past 3 years *agouti* (Bultman et al. 1992), *obese* (Zhang et al. 1994), and *fat* (Naggert et al. 1995) have been cloned in mice, and their human homologs identified. Direct mutational analysis of the coding sequence for the human obese (OB) gene has failed to detect any mutation in a limited number of obese humans (Considine et al. 1995). Plasma concentrations of leptin are highly correlated with percentage of body fat (Maffei et al. 1995; Considine et al. 1996). However, there is considerable variability in leptin concentrations for any percentage of body fat, suggesting that some individuals could be resistant to leptin's effects (Maffei et al. 1995).

The cloning of the OB receptor (OBR) (Tartaglia et al. 1995) and the demonstration that mutations of this gene are responsible for the *diabetes* (Obr^{db}) phenotype in mice (Cheng et al. 1996; Chua et al. 1996) and the *fatty* Obr^{fa} phenotype in rats (Chua et al. 1996) allows for genetic linkage testing by means of sib-pair analysis, para-

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metric linkage, or association studies of the possible role of this gene in human obesity by the analysis of segregation of polymorphic markers closely linked to OBR. To this end, we have mapped OBR physically by radiation hybrid (RH) mapping and placement on a contig composed of 10 adjacent yeast artificial chromosomes (YACs) and 5 P1 artificial chromosome (PACs). The location of the human homolog of Obr was predicted to be on 1p based on conserved linkage of most of the telomeric half of mouse chromosome 4 with human 1p (Blank et al. 1991; Abbott et al. 1992). Although OBR does map to 1p, there has been an apparent rearrangement of the order of these genes in humans relative to that observed in mice and rats.

RESULTS

Using the Gene Bridge 4 RH panel, OBR maps to 0.9 cR centromeric of WI-9515 and 2.2 cR telomeric of WI-7249, PGM1 maps to the same location as D1S230, and D1S85 maps to the same location as WI-9848, all with a lod >3 (Fig. 1). Using the Stanford panel, PGM1 maps to the same location as D1S515, and D1S85 maps 9.21 centirads (cR) telomeric of D1S476 and 20.93 cR centromeric of D1S220, all with a lod >8. OBR could not be mapped on the Stanford panel with a lod >6 likely because of an insufficient density of markers included within the framework map (as is the case for 50% of markers submitted). Based on the RH mapping, the gene order in humans is Cen–//–OBR–PGM1–D1S85.

On the basis of our assessment of the sequence-tagged site (STS) content of the 10 YACs positive for OBR [895B12 (1160 kb), 967A10 (470 kb), 940H12 (480kb), 930F4 (1050 kb, 1270 kb), 817D6 (1590 kb), 855A3 (1030 kb), 785A11 (1560 kb), 822B4 (1150 kb), 912G11 (1590 kb), and 946B12 (1500 kb)] the 3′ end of OBR should lie between WI-7249 and WI-9515 (Fig. 1). Three PACs (9915, 9916, 9917) were identified that contained the 3′ end of OBR (OBR–STS from bp 3064–3321) and two PACs (10353, 10354) that contained the 5′ end of OBR (OBR–5′ from bp 426–558). Of the five PACs, only two (10354, 9915) were positive for sequence from the middle of OBR (OBR–mid from bp 1250–1489), allowing alignment of the PACs (Fig. 1). Only two of the PACs (9916, 9917) contained the STS WI-5182, thereby placing WI-5182 within a 120-kb region 3′ of OBR. None of the eight STSs mapped on the YACs were positive for any of the five PACs.

Five PGM1-positive YACs [948F3 (890 kb), 677D7 (790 kb), 951E8 (1740 kb), 717C6 (480 kb, 770kb), 757C5 (870 kb)] and four D1S85 positive YACs [812D6 (1260 kb), 799H3 (860 kb), 911H5 (1320 kb), 904G5 (1670 kb)] were identified. In addition, three definitively positive complement 8 β (C8B) YACs (935D9, 789E12, and 728B4) and six definitively positive JUNC YACs (904H5, 789E12, 766B1, 719A9, 726B12, and 757F7) have been identified by the Whitehead Institute (Whitehead 1995). Based on the physical map including the YAC contigs constructed at the Whitehead, the gene order in humans is Cen–//–OBR–PGM1–JUNC–D1S85–C8B.

Genetic mapping using the human families was performed to confirm the physical maps and produced the map shown in Figure 1. The gene order on the basis of the genetic map is Cen–//–PGM1–D1S85.

DISCUSSION

OBR maps to 1p in the interval between WI-7249

Figure 1 Radiation hybrid, physical, and genetic maps in the vicinity of OBR. Radiation hybrid map: The upper radiation hybrid map was derived using the Stanford Human Genome Center (SHGC) 3 RH panel. Only markers with lod >6 are shown. Raw data were submitted to SHGC as UNK27384-00001 (OBR), UNK21531-00001 (PGM1), and UNK00512-00001 (D1S85). The lower radiation hybrid map was derived using the Genebridge 4 RH panel, and all markers with lod >3 in the interval are shown. Raw data were submitted to WICGR as Jan7 12:13:26 (OBR), Jan26 14:25:56 (PGM1), and Jan26 14:24:46 (D1S85). Distances in cR are indicated above the maps. Physical map: Three contigs encompassing OBR, PGM1, and JUNC/D1S85/C8B are shown. YACs for OBR, PGM1, and D1S85 were identified by screening the CEPH Mega YAC library, enabling placement of these markers relative to the framework Whitehead map. The marker order around PGM1 and JUNC/D1S85/C8B is based on the most current Whitehead release (Whitehead 1995). The marker order around OBR is based on determined STS content. Five PACs are also shown. YACs are identified by address below the map, and sizes are indicated in parentheses. Genetic map: Unresolvable markers are listed above each other. Distances are indicated in centiMorgans (cM).

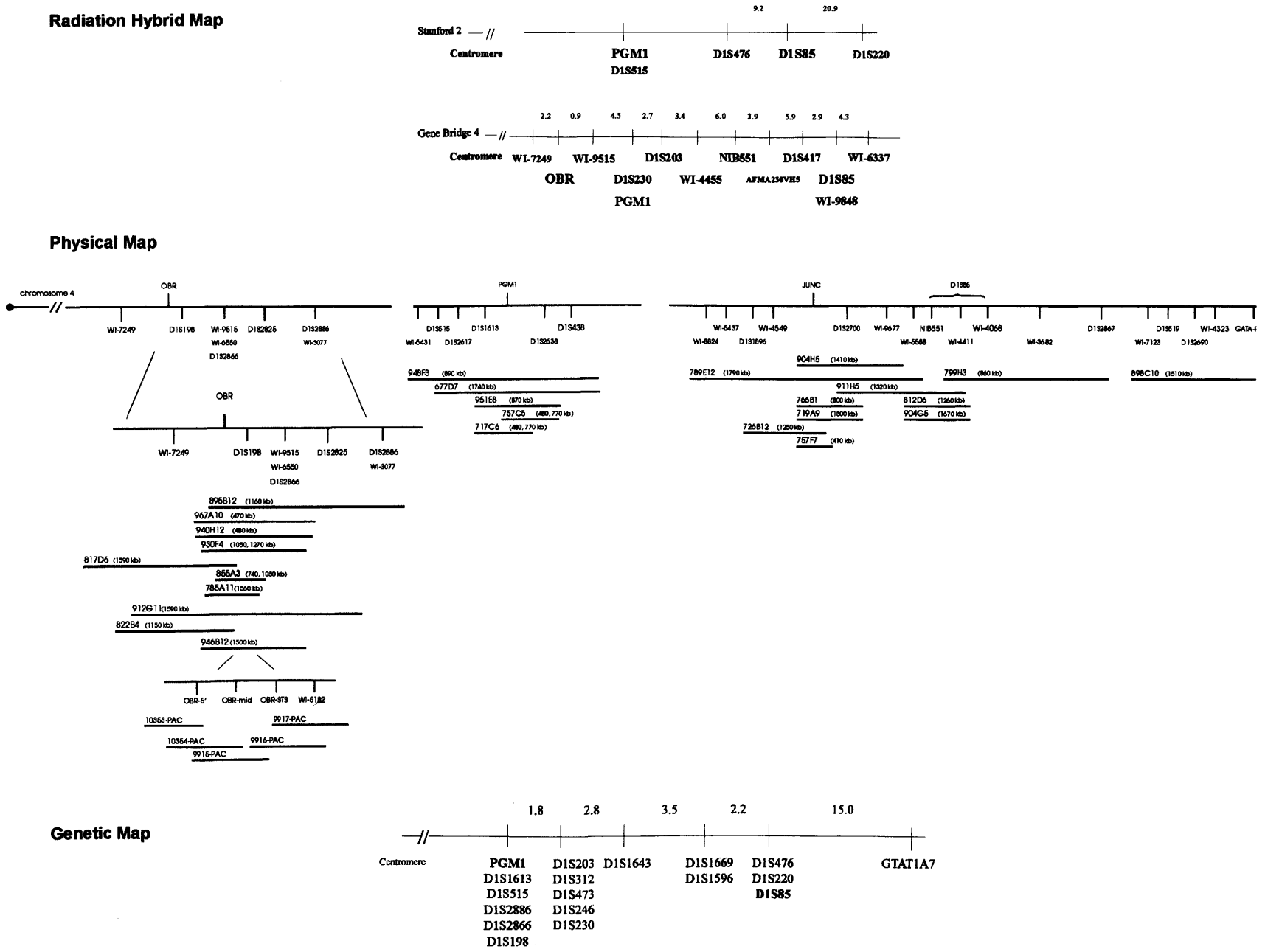

Figure 1 (*See facing page for legend.*)

and WI-9515. WI-5182 maps within 120 kb 3′ of OBR.

The RH, physical (YAC), and genetic maps agree both in order and relative magnitude of interlocus intervals with each other and largely with those published previously (Cohen et al. 1993; Gyapay et al. 1994; Murray et al. 1994; Whitehead 1995). The only discrepancy in the maps is with the physical YAC map from the Whitehead Institute (Whitehead 1995). According to the Whitehead map, D1S476 is telomeric of D1S85; however, our RH map places D1S476 centromeric of D1S85. Additionally, the marker order indicated by the Whitehead map in the vicinity of OBR is inconsistent with the physical map reported here. We detected all of the definitively positive STSs reported by the Whitehead; however, we also detected many additional STSs in these YACs that do not agree with either the ambiguous hits or the inferred hits based on fingerprinting or STS content data. Because the data reported here are based on unambiguously positive and negative STS content, the map presented here is more likely to be correct.

OBR likely maps to 1p31 or 1p22 between PGM1 and D1S22. The definitive cytogenic assignment of OBR is difficult without in situ data because both PGM1 and D1S22 have been reported to map to the adjacent cytogenetic bands 1p31 and 1p22 (Dracopoli et al. 1988, 1991; Whitehouse et al. 1992; Dracopoli et al. 1994).

On the basis of the rodent maps for the regions surrounding Obr^{db} and Obr^{fa}, OBR would have been predicted to lie between D1S85 and PGM1 (Fig. 2). However, it appears that the gene order on this segment of 1p has not been conserved from rodent (Cen–//–Jun–Pgm1–Obr^{db}/Obr^{fa}–D4H1S85–C8b) to human (Cen–//–OBR–PGM1–D1S85–C8B). There is an inversion of the Obr^{db}/Obr^{fa} region centromeric of Jun and be-

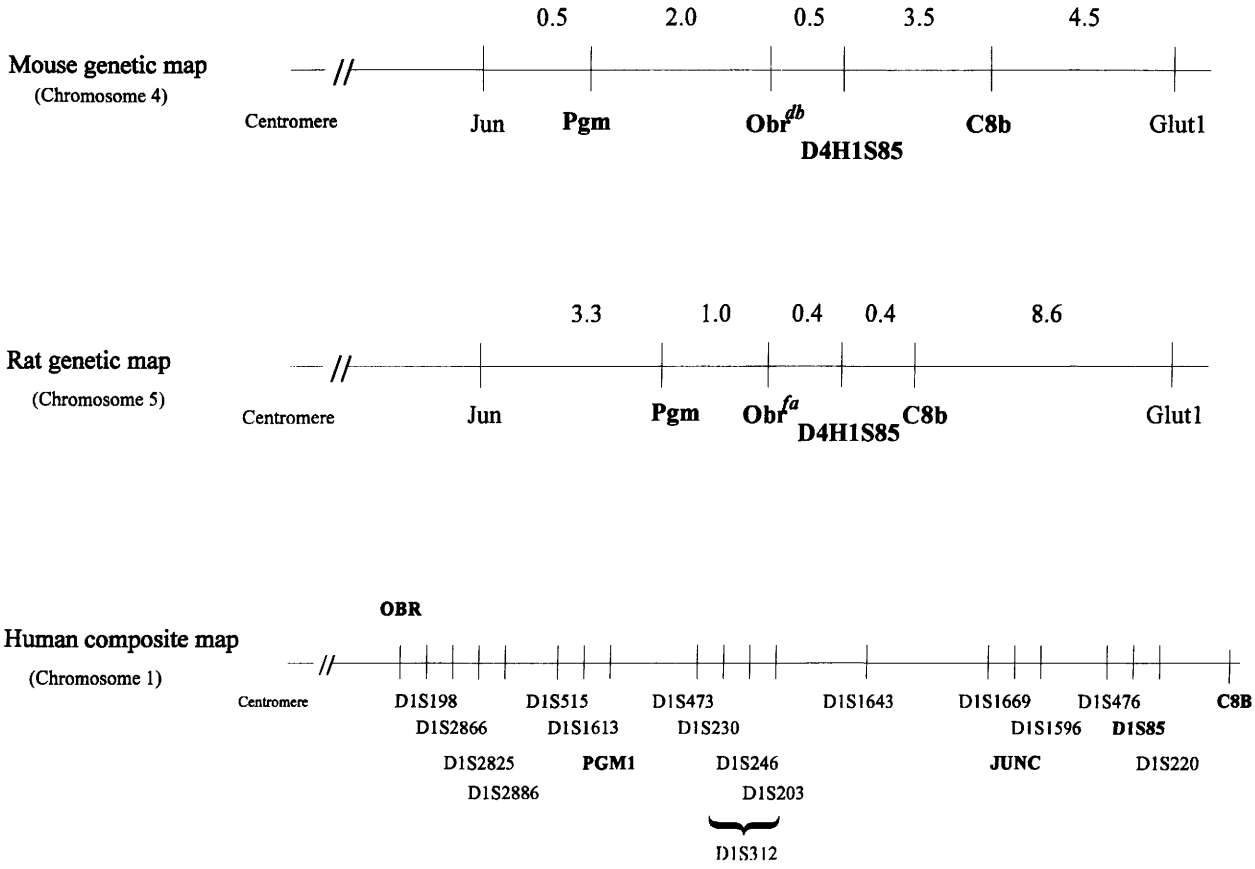

Figure 2 Comparison of murine chromosome 4, rat chromosome 5, and human chromosome 1p. The mouse and rat genetic maps around Obr^{db} and Obr^{fa} are indicated above, and the human composite map of the syntenic region based on RH, physical YAC, and genetic maps is indicated below. Distances for the rodent maps are in cM.

tween Obr and D4H1S85 to produce this order in human. It had been assumed previously that mouse chromosome 4 represented a conserved linkage group of >20 cM with human 1p (Nadeau 1989), but it now appears that this segment is only syntenic, because the gene order has not been conserved. Although Glut1, C8b, D4H1S85, Pgm, and Jun have been mapped in the mouse and rat, and are conserved in order although not in absolute distance, such order need not be conserved in humans. Such a break in marker order in a syntenic region has been demonstrated previously on mouse chromosome 5 in the region surrounding the Huntington's disease gene on human chromosome 4 (Grosson et al. 1994).

Now that the position of OBR has been determined, polymorphic molecular markers such as D1S2866 and D1S198 can be used in linkage studies to test the hypothesis that this gene is playing a role in the genetic basis of human obesity.

METHODS

PCR

PCRs were performed in 20-μl volumes containing 75 ng of genomic DNA, 2 pmoles of both forward and reverse end-labeled primers or 100 ng of both forward and reverse unlabeled primers, 200 mM each dNTP, 1 unit of *Taq* DNA polymerase, and 1× *Taq* buffer consisting of 10 mM Tris (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, and 0.1 mg/ml of gelatin. Unless otherwise indicated, cycling conditions consisted of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 30 sec for 35 cycles.

RH Mapping

Fifty nanograms of the Gene Bridge 4 (Walter et al. 1994) and Stanford 3 RH (Stanford) panels was tested in triplicate for the ability to amplify a product using PCR with primers specific to OBR, PGM1, or D1S85 (Table 1). Products were visualized with ethidium bromide after electrophoresis through a 2% agarose gel. Cell lines that did not produce concordant results for the three assays were designated ambiguous. Radiation hybrid servers at the Whitehead Genome Center (http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl) and Stanford Human Genome Center (http://shgc@stanford.edu) were used to map these genes relative to the framework maps. Linkage with a lod >3 was reported from the Whitehead Institute, whereas the Stanford Human Genome Center reported linkage only with markers producing a lod >6. lod score thresholds were determined by the individual genome centers based on their local experiences with the radiation hybrid panels.

YAC and PAC Mapping

On the basis of the location of OBR by RH mapping, all YACs in the Whitehead data base (Whitehead 1995) mapping to the 5 cM interval surrounding the RH location were tested for OBR by boiling 2 μl of washed yeast cultures for 5 min followed by PCR assay with OBR–STS using standard conditions used for the RH mapping. In addition, the Centre d'Etude du Polymorphisme Humain (CEPH) Mega YAC library (Dausset et al. 1992) was screened by PCR at the Baylor College of Medicine Human Genome Center using the primers OBR–STS. The OBR-positive YACs were tested for eight markers [four microsatellites and four expressed sequence tags (ESTs)] reported to map to the interval previously (Whitehead 1995). The YACs were then aligned by STS content. YACs that did not contain any STS in addition to OBR were assumed to be chimeric and are not shown.

The CEPH Mega YAC library (Dausset et al. 1992) was screened by PCR for PGM1 and D1S85 using primers PGM1–STS and D1S85–STS.

The PAC library (Ioannou et al. 1994) was screened by PCR with the primers OBR–STS and OBR–5′. PAC DNA was recovered using a standard plasmid isolation protocol (Birnboim and Doly 1979). PAC STS content of D1S198, D1S2886, D1S2866, D1S2825, WI-5182, WI-7249, WI-9515, WI-6550, WI-3077, OBR-mid (*F*, 5′-ATCTATAAGAAGGAAAACAAGAATGT, bp 1250–1275; *R*, 5′-GATATTGACATCAATCACTATAATTC, bp 1489–1463; GenBank accession no. U43168) and *OBR*–5′ (*F*, 5′-ACTTTTCTAACTTATCCAAAACAACTT, bp 426–452; *R*, 5′-TGTTCAAAAACTAAAGAATTTACTGTT, bp 558–532; GenBank accession no. U43168) was determined by using 50 ng of purified PAC DNA in an otherwise standard PCR reaction.

Genetic Mapping

Genetic mapping of 18 markers including two loci mapped previously relative to Obr^{db} and Obr^{fa} (D1S85 and PGM1) was performed utilizing pedigrees collected by the Venezuela–United States Collaborative Research Group to confirm the marker order derived from the RH and YAC maps and to integrate a greater number of genetic markers into the map. One three-generation family consisting of 52 individuals and another two-generation family consisting of 16 individuals were used for all genetic analyses. All individuals were typed for all markers.

For genetic markers visualized by autoradiography, 100 pmoles of both forward and reverse primer were end-labeled in a 25-μl reaction volume containing 10 m of [γ-^{32}P]ATP, 1× polynucleotide kinase buffer (New England Biolabs, Beverly, MA), and 20 units of polynucleotide kinase for 30 min at 37°C. Radiolabeled PCR products were electrophoresed on a 43-cm nondenaturing 6% polyacrylamide gel, dried, and exposed to film. Unlabeled PCR products (D1S85 and PGM1) were electrophoresed on agarose gels stained with ethidium bromide.

All gels were read by two individuals for accuracy and consistency. Any potential genotyping errors indicated by closely located double recombinations were resolved by retyping questionable individuals.

Genetic Mapping Analysis

GTAT1A7, D1S85, and PGM1 were chosen as initial anchors for the genetic map. Additional markers were se-

Table 1. PCR Assay Specification

Marker	Primer sequence	PCR conditions	Product size	Electrophoresis	Detection	Reference
OBR-STS (bp 3064-3089) (bp 3297-3321)	F: 5'CAGTTCAACAGTGTTAACTTCTCT R: 5'TGCTGATCTGATAATATAAAAAATG	94°C 30 seconds 53°C 30 seconds 72°C 30 seconds	257 bp	2% agarose	ethidium bromide	Tartaglia et al. 1995
PGM1-STS	F: 5'TAGGTGGAGCATCAGGAGTG R: 5'AAATGGGGGGAAAGATTCAC	standard conditions	104 bp	2% agarose	ethidium bromide	Whitehouse et al. 1992
D1S85-STS	F: 5'CATGTGTCTTAACCACTCTGAACC R: 5'CTATGGGGATAGGGATAGGGATTG	standard conditions	280 bp	2% agarose	ethidium bromide	Verga et al. 1989
D1S85-polymorphic	F: 5' GAATCCCTCTTATGGTGTTCGA R: 5' TCAGAGTGGTTAAGACACATG	94°C 1 minute 58°C 2 minutes 72°C 2 minutes	1.35 0.85 kb	1.5% agarose gel	ethidium bromide	Verga et al. 1989
PGM1-polymorphic	F: 5' AAGCTTCTCTCTATGTCTTCCTCAG R: 5' GCCCGCAGGTCCTCTTTCCCTCACA	94°C 30 seconds 63°C 30 seconds 72°C 45 seconds Restrict product with RsaI	317 bp	3% agarose 2% low melt agarose gel	ethidium bromide	Whitehouse et al. 1992
GTAT1A7, D1S476, D1S220, D1S1669, D1S1596, D1S1643, D1S203, D1S312, D1S473, D1S246, D1S230, D1S1613, D1S515, D1S2886, D1S2866, D1S198	unmodified from previously published sequence	standard conditions		6% non-denaturing polyacrylamide gel	radio-isotope	Gyapay et al. 1994; Murray et al. 1994
WI-7249, WI-9515, WI-6550, WI-3077, WI-5182	unmodified from previously published sequence	standard conditions		2% agarose	ethidium bromide	Whitehead 1995

quentially added to the map using the CRI-MAP option "all" (CRI-MAP, v. 2.4). The final map was then checked for accuracy by "flipping" the orders of sequential markers as many as seven at a time to ensure that there was not a more likely order of markers than tested previously. The maximum likelihood estimates of the recombination fractions between markers were calculated using the "chrompic" function.

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